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Case Report

Treatment of haemoperitoneum secondary to ruptured granulosa cell tumours in two mares

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Present addresses: ¹Dick Vet. Equine Hospital, R(D)SVS, Roslin, Midlothian, UK; ²Equine Surgical Referrals, Cheam, Surrey, UK; ³Dierenkliniek De Bosdreef, Moerbeke-Waas, Belgium**Keywords:** horse; granulosa cell tumour; ovariectomy; haemoperitoneum; autologous blood transfusion; abdominocentesis

Summary

This report describes two cases of successful surgical management of granulosa cell tumours (GCT) in mares presenting with haemoperitoneum (HP). Controlled abdominal drainage was initially attempted in Case 1 but was not successful. A ventral midline exploratory laparotomy allowed removal of a haemorrhaging 13 kg GCT. The mare made a full recovery and returned to normal work as a driving pony 11 months post-operatively. In Case 2 controlled abdominal drainage was followed by standing left flank laparoscopic visualisation of the bleeding ovary and transection of the ovarian pedicle by electrocautery. The GCT was then removed via a ventral midline incision due to its large size. Haemoperitoneum can be associated with GCTs and in some cases is severe enough to prompt emergency treatment. Stabilisation of the patient and removal of the haemorrhaging GCT can lead to a successful outcome.

Introduction

Haemoperitoneum (HP) is uncommon in horses (Dechant *et al.* 2006) and its investigation can be difficult owing to the array of causes and the nature of their manifestation (Dechant *et al.* 2006; Conwell *et al.* 2010). Causes of HP in horses include splenic, hepatic or uterine rupture, broad ligament haemorrhage, ovarian haematomas, abdominal neoplasia (primary or metastatic), rupture of mesenteric arteries secondary to strongyle migration, as well as vascular leakage associated with the renal artery, ileocaecal artery and aorta (Dechant *et al.* 2006; Conwell *et al.* 2010).

Ovarian tumours account for 2.5% of all neoplasms in the horse (Sundberg *et al.* 1977) and granulosa cell tumour (GCT) is the most common neoplasm affecting the equine ovary (McCue *et al.* 2006). The most common clinical signs associated with ovarian GCTs are changes in behaviour, specifically developing aggressive, stallion-like or nymphomania behaviour, infertility, anoestrous or colic (Crabtree 2011). It is uncommon that mares with GCTs present with secondary HP, and only seven cases have been previously reported in the literature (Green *et al.* 1988; Gatewood *et al.* 1990; Alexander *et al.* 2004; Dechant *et al.* 2006; Harper *et al.* 2010) with only five of those surviving. This case report describes the clinical signs and management of

two mares presenting with severe HP secondary to GCTs which were successfully treated by ovariectomy.

Case 1 details

A 19-year-old (272 kg) Pony mare used for driving was referred with a history of abdominal distention, inappetence, dullness and lethargy of 1 month duration. The mare was reported to have recently displayed abnormal oestrus cycle behaviour.

Clinical investigations

On presentation at the hospital the mare was quiet, with marked abdominal distention. She was tachycardic (96 beats/min) and had weak peripheral pulses. The rectal temperature was lower than normal (36.2°C). Mucous membranes were very pale with a capillary refill time of 3 s. The mare's peripheral blood packed cell volume was 11%, the red blood cell count was 2.05×10^{12} cells/L (reference range $6.8\text{--}12.9 \times 10^{12}$ cells/L), total protein was 50 g/L and lactate was >9 mmol/L.

Percutaneous abdominal ultrasonography with a GETM 4C-RS probe (2.0–5.0 Hz 60 mm radius convex probe) revealed large areas of mixed echogenicity with a free swirling appearance (Fig 1) consistent with HP (Harper *et al.* 2010). A large mass of mixed echogenicity (Fig 2) which had a multilocular 'honeycomb' appearance typical of a GCT (White and Allen 1985) was identified. The mass was approximately 20 × 30 cm in size and was visible from both the left and right paralumbar fossae. Abdominocentesis yielded haemorrhagic fluid with a packed cell volume of 40%, a total protein of 56 g/L and total white blood cell count 12×10^9 cells/L which confirmed the presence of a HP. Per rectum examination was attempted but the mare resented the procedure. Following administration of hyoscine butylbromide (0.3 mg/kg bwt i.v., Buscopan²) to facilitate per rectum examination, the mare collapsed and thus per rectum examination was not completed.

Emergency i.v. fluid resuscitation was initiated. The pony received a 10 L bolus of isotonic crystalloid solution (Aquapharm 11, solution for infusion, compound sodium lactate i.v. infusion BP³) and an allogenic blood transfusion of 8 L of fresh whole blood (not cross matched) from two hospital donor mares. The mare's condition stabilised and she was standing again by approximately 10 min following fluid



Fig 1: Transcutaneous abdominal ultrasound image from Case 1. Large areas of mixed echogenicity with a free swirling fluid appearance, consistent with haemoperitoneum.

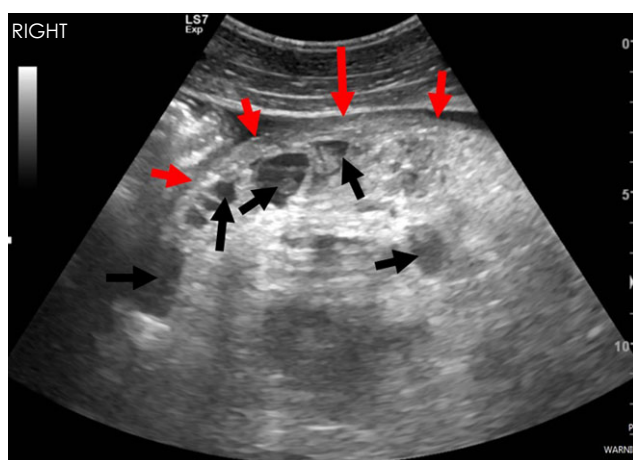


Fig 2: Case 1: Transcutaneous abdominal ultrasound image via the right paralumbar fossa. A large ovarian mass of mixed echogenicity with hypoechoic cystic structures (black arrows) adjacent to homogeneous soft tissue stroma consistent with a multilocular 'honeycomb' appearance typical of a GCT. Red arrows delineate the border of the mass.

and blood administration. Controlled blood collection from the abdominal cavity was attempted following abdominocentesis with a metal 14 gauge 5 cm teat cannula; however, the procedure was aborted due to poor rate of fluid collection and repeated occlusion of the cannula. The mare's parameters improved over an hour following blood transfusion and fluid resuscitation; HR 68 beats/min, PCV 16% and pale pink mucous membranes.

Owing to the appearance of an abnormal ovary and history of abnormal behaviour, GCT or ovarian haematoma were suspected. Although other sources of HP could not be ruled out, a working diagnosis of severe HP possibly associated with a suspected GCT was made.

Treatment

Owing to the fatal risk of ongoing haemorrhage, emergency exploratory laparotomy was performed. The mare was

administered flunixin meglumine (1.1 mg/kg bwt i.v. b.i.d., Meflosyl⁴) and ceftiofur (2.2 mg/kg bwt i.m. b.i.d., Excenel⁴) pre-operatively. A cephalosporin was chosen over the standard hospital protocol of a penicillin and aminoglycoside to provide a broad spectrum of cover and minimise any nephrotoxic effects due to the hypovolaemic state of the pony.

Following induction of general anaesthesia, the mare was positioned in dorsal recumbency for exploratory laparotomy and the ventral abdomen was aseptically prepared for surgery. A 25 cm ventral midline laparotomy incision was made from the umbilicus cranially. Following digital penetration of the peritoneum a substantial volume of sanguinous fluid (approximately 9 L) containing blood clots was spontaneously released. Suction was instigated to aid visualisation within the abdominal cavity. A large, 40 × 30 × 25 cm mass with a firm, multi-nodular texture was palpable, occupying much of the caudal abdomen. Partial exteriorisation of the mass revealed the most likely source of profuse haemorrhage to be a capsular tear. The mass was noted to be associated with the right ovary. The right ovarian pedicle was ligated with a combination of transfixing, circumferential ligatures (4M braided-lactomer [Polysorb]⁵) and electrocautery applied with a LigasureTM Vessel Sealing System (Ligasure Atlas⁵). The 13 kg mass was removed (**Fig 3**). The abdominal cavity was lavaged with 15 L of sterile polyionic fluid, and as much fluid as possible was then removed with suction post-lavage. No other abnormal findings were found intra-abdominally and the laparotomy incision was closed with a routine three-layer closure. Intraoperatively the mare received a 6 L fresh whole blood transfusion (un-matched) from two hospital donor mares, 1 L bolus of colloids (Geloplasma⁶), isotonic crystalloid solution (4 mL/kg/h Aquapharm 11³) and 1 L bolus of 7.2% sodium chloride hypertonic saline (Vetivex 20⁷). The mare recovered uneventfully from general anaesthesia and isotonic crystalloid i.v. fluid therapy (4 mL/kg/h Aquapharm 11³) was continued for 24 h post-operatively. Histopathological examination of the tissue sections from the mass confirmed the diagnosis of a macrofollicular granulosa cell tumour.

Post-operative progress

Post-operative analgesia was provided by administration of flunixin meglumine (0.5 mg/kg bwt i.v. b.i.d., Meflosyl⁴) for 5 days. Post-operative antimicrobial therapy consisted of ceftiofur (2.2 mg/kg bwt i.m. b.i.d., Excenel⁴) for 5 days.

The mare showed steady improvement and was discharged 13 days following surgery. At discharge the packed cell volume was 17%, total protein was 65 g/L and systemic lactate was 0.8 mmol/L. A follow-up phone call with the owner 11 months post-operatively revealed the mare to be doing well and back in work as a driving pony.

Case 2 details

A 10-year-old Thoroughbred mare was presented for investigation of increasing abdominal distention which had been noted over the previous 4 weeks. The mare was reported to be more aggressive than usual but the owners were unsure of any changes to her oestrus cycle. Clinical examination revealed a normal heart rate, rectal temperature and respiratory rate but poor body condition with an enlarged abdomen.

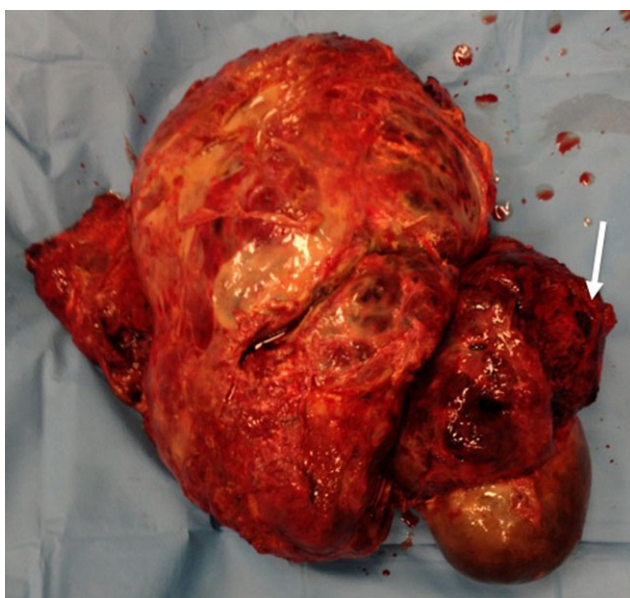


Fig 3: Case 1: following mass removal, an incision was made into the GCT (white arrow) revealing haemorrhage within the mass associated with the capsule rupture.

Investigations

Examination per rectum revealed a large (circa 40 cm diameter), smooth, hard mass in the right ventral abdomen, thought to be the right ovary. All aspects of the mass could not be palpated per rectum due to its size. Ultrasound examination findings per rectum revealed the mass to be of heterogeneous echogenicity with multiple areas of hypoechoic fluid contained within honeycomb-like soft tissue stroma and additional areas of homogeneous soft tissue echogenicity (**Fig 4**) surrounded by an excessive amount of hypoechoic peritoneal fluid. Percutaneous ultrasonographic examination over the right flank region and ventral abdomen confirmed a markedly increased amount of peritoneal fluid and a similar poly-cystic structure within the mass.

Routine haematology revealed mild anaemia (RBC 6.0×10^{12} cells/L, HCT 27.7%, HGB 10.1 g/dL), and mild leukopenia (5.3×10^9 /L) with lymphopenia (1.22×10^9 cells/L). Needle centesis of the peritoneal cavity was performed from the ventral midline using a 19 gauge 38 mm needle. Sero-sanguinous fluid jetted out of the needle under pressure. Analysis of the fluid revealed a total protein of 30 g/L, red blood cell count of 0.39×10^{12} cells/L and total white blood cell count of 0.69×10^9 cells/L. A packed cell volume value was not provided by the analyser.

Treatment

Under sedation (romifidine 0.08 mg/kg bwt i.v., Sedivet², plus butorphanol 0.01 mg/kg bwt i.v., Torbugesic⁴), the skin of the ventro-cranial abdomen was prepared for aseptic surgery and a 5 mm stab incision was made using a no. 11 blade through the skin, sub-cutis and linea alba before blunt introduction of a 10 cm long metal teat cannula through the peritoneum in the midline. Approximately 30 L of serosanguinous fluid were collected over 4 h. The horse's heart rate was monitored during fluid collection to ensure it did not increase. Drainage was discontinued when the flow

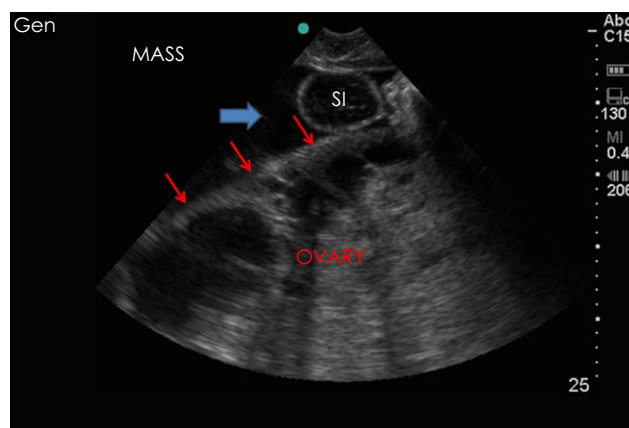


Fig 4: Case 2: Ultrasound image obtained per rectum of the ovarian mass with hypoechoic cystic structures and more homogeneous soft tissue stroma within it. Red arrows delineate the dorsal border of the mass, the excessive volume of hypoechoic peritoneal fluid (blue arrow) is also visible. SI = small intestinal loop.

rate appeared to decrease to a slow drip; ultrasound examination was not repeated.

After a 24-h period of starvation, the mare underwent laparoscopic surgery under standing sedation with romifidine (0.08 mg/kg bwt i.v., Sedivet²), plus butorphanol (0.01 mg/kg bwt i.v., Torbugesic⁴). Perioperative antibiotics and NSAIDs were administered 2 h before surgery (gentamicin 6.6 mg/kg bwt i.v. s.i.d., Genta Equine⁷, procaine penicillin 15 mg/kg bwt i.m. b.i.d., Depocillin⁸, flunixin meglumine 1.1 mg/kg bwt i.v., Flunixin⁹). Portals were made in a triangular formation. The laparoscope portal was made at the level of the ventral aspect of the right tuber coxa, immediately caudal to the last rib. The second (LigasureTM) portal was made at the same level dorso-ventrally, but 10 cm caudal to the laparoscope portal. The third (instrument) portal was made 10 cm vertically below the laparoscope portal. All three portal sites were anaesthetised using direct infiltration of 20 mL 2% lignocaine (Lidocaine injection¹⁰) at each site and 12 mm diameter laparoscope trocars (VersaportTM V2 5 mm–12 mm Long Trocar with Fixation Cannula and Universal Seal⁵) were inserted in a standard fashion (Lloyd *et al.* 2007).

A 57 cm long 10 mm diameter 30° forward oblique viewing rigid laparoscope was introduced into the abdominal cavity. An abnormally large volume of sero-sanguinous peritoneal fluid was visible within the peritoneal cavity indicating either that the abdomen had not been drained significantly, or that the effusion had reformed in the interim 24 h. Insufflation of the abdomen with carbon dioxide was not performed as there was adequate visualisation of the ovarian pedicle. Only the dorsal aspect of the enlarged right ovary could be visualised. The ovarian pedicle was injected with 20 mLs 2% lignocaine (Lidocaine injection¹⁰) before being cauterised and transected using the LigasureTM Vessel Sealing System (Ligasure Atlas⁵). The pedicle was very thick and the ligasure forceps were used to bluntly dissect it into lateral and medial sections prior to each cauterising bite, because the full thickness of the pedicle was too large for the forceps. Once the ovary was completely free of attachments within the abdomen, the laparoscopic trocars

were removed and portals closed in a routine fashion (Lloyd *et al.* 2007).

General anaesthesia was then induced and the mare was positioned in dorsal recumbency. A 30 cm long ventral midline laparotomy incision was made at the level of the umbilicus and directed cranially to allow abdominal exploration. The right ovary was exteriorised via this incision and a rupture of the ovarian capsule was evident (**Fig 5**). Sectioning of the exteriorised ovary confirmed fresh haemorrhage and a haematoma adjacent to the area of the ruptured capsule (**Fig 6**). As there was still a copious volume of fluid within the abdomen, a 32F thoracic drain (standard trocar catheter¹¹) was placed 10 cm lateral to the cranial aspect of the laparotomy incision with the finger of a surgical glove (with a slit at the distal end) sutured over its end to act as a one-way valve for egress of abdominal fluid. The laparotomy incision was closed routinely in three layers, and a stent was placed for recovery.

Post-operative progress

The mare recovered uneventfully from surgery and the abdominal drain was removed 48 h post-operatively. She was discharged from the hospital 7 days after surgery. Follow-up examination 6 weeks post-operatively revealed that the mare was clinically well. Histological analysis of the excised ovary confirmed a GCT.

Discussion

Pathogenesis

Active haemorrhage from GCT capsule rupture has been grossly confirmed as the cause of HP during laparotomy, laparoscopy or post-mortem in horses (Gatewood *et al.* 1990; Alexander *et al.* 2004; Harper *et al.* 2010; Sherlock *et al.* 2016). We suggest that rapid growth of the ovary may predispose to rupture of the ovarian capsule and haemorrhage however the cause is unclear. Both the GCTs in this report were large; however, GCTs of similar size have been described in the literature without associated bleeding (Lloyd *et al.* 2007). In a previous case report, haemorrhaging GCTs weighing 4 and 8 kg were removed from mares with HP, but size measurements were not noted (Alexander *et al.*



Fig 5: Case 2: GCT capsule rupture; suspected to be the source of haemorrhage.

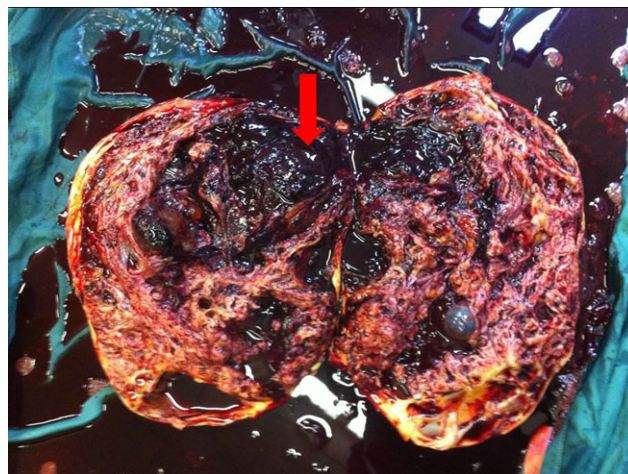


Fig 6: Case 2: excised ovary sectioned, revealing fresh blood and haematoma formation adjacent to the capsular rupture (arrow).

2004). Three cases of HP associated with haemorrhaging GCTs have also been reported by Dechant *et al.* (2006) although more specific details of these cases were not given. Although the risk of HP from GCTs appears to be low, early detection and surgical removal of GCTs is recommended to avoid this potentially life-threatening sequel. In women an increase in follicle-stimulating hormone (FSH) receptor expression has been reported in the vascular endothelia on the periphery of tumours. The FSH binding these receptors in turn is thought to promote neovascularisation (Radu *et al.* 2010). Increased FSH receptor expression resulting in increased neovascularisation in the periphery of a tumour may be associated with severe haemorrhage caused by capsular tears in larger GCTs in mares, although what instigates the capsular tear is unknown.

Presentation and emergency stabilisation

This report highlights the fact that GCTs may occasionally present with HP and, that in some cases, may require prompt medical resuscitation and emergency surgical intervention for a successful outcome, which, to the authors' knowledge, has not previously been reported. Given the size of the tumours in this case report, it is likely that they had been present for much longer than the time period that clinical signs had been noted by the owners. These two cases presented with signs attributable to the HP i.e. anaemia and abdominal distension, and Case 1 also presented with collapse. This was in contrast to the typical behavioural signs that are more commonly present in mares with GCT, and which are attributable to the hormonal imbalances associated with these tumours. Identifying the cause of HP can sometimes be challenging, but in both cases in this report the presence of a very large, abnormal intra-abdominal mass was indicative of the instigating cause of haemorrhage.

A previous case series described sub-acute intra-abdominal haemorrhage associated with GCTs in two mares (Alexander *et al.* 2004). Surgical treatment was performed electively following medical stabilisation for 10–21 days. Although this elective approach might be preferred, intra-abdominal haemorrhage can be rapid and severe in some cases, and can be successfully managed with prompt

surgical excision of bleeding GCTs. Acute emergency surgical intervention was undertaken in Case 1 which presented with severe anaemia and collapse because of the concern that continued haemorrhage in an animal with an already low packed cell volume might result in fatal cardiovascular compromise.

Stabilisation of hypovolaemic cases secondary to HP can include allogenic or autogenous blood transfusions, fluid therapy and antifibrinolytic agents such as aminocaproic acid. General guidelines for performing allogenic fresh whole blood transfusions include typing both the donor and recipient blood. Both major and minor cross matching is recommended (Hart 2011), but this is difficult in practice. Fresh blood was collected separately from two donor horses pre-surgery to be administered pre- and intraoperatively and a further collection was taken and administered intraoperatively from a third donor horse. No cross matching was performed prior to the three fresh whole blood transfusions as discussed by Durham (1996). Ideally cross matching should be performed for subsequent transfusions after the first unmatched transfusion in an emergency (Hart 2011). Cross matching was performed post-operatively on Case 1 to identify suitable donors for any subsequent allogenic transfusions if required. Although in an emergency situation unmatched fresh whole blood may be used for the first transfusion subsequent blood for transfusions should be cross matched to prevent transfusion reactions (Durham 1996). The blood for transfusion was collected from hospital teaching mares as these were the only donors available; it is recommended that blood is taken from geldings to minimise reactions occurring (Sellon and Wise 2010). Autotransfusion of abdominal blood collected from cases with HP has been described (Waguespack *et al.* 2001; Finding *et al.* 2011; Fouché *et al.* 2014). However, it is contraindicated if the HP is associated with neoplasia (Finding *et al.* 2011) or on-going haemorrhage (Hart 2011), and intraperitoneal blood is time consuming to collect, therefore it may not be suitable for unstable cases requiring prompt surgical treatment (Case 1). HP has been reported to have a high association with neoplastic causes (Dechant *et al.* 2006; Conwell *et al.* 2010) and therefore, the sources of HP should be investigated before autotransfusion is elected.

In both cases a significant volume of HP was present, indicated by ultrasound findings. A subjective estimate of between 5 and 10 L was made for Case 1 (bwt 272 kg) following ultrasound examination and approximately 9 L of blood was obtained at surgery, but it is likely that more blood was present in the depths of the abdomen. In Case 2 approximately 30 L of serosanguinous fluid was collected from the abdomen prior to laparoscopy. Drainage of the HP was only partially effective in Case 2 and unsuccessful in Case 1, probably due to blood clots blocking the drainage cannula. If possible, controlled drainage is recommended before general anaesthesia and exploratory laparotomy to improve case stability and surgical visibility (Waguespack *et al.* 2001; Conwell *et al.* 2010; Finding *et al.* 2011).

Treatment options

The reported prognosis for survival following medical management of horses with HP is very variable (17–84%, Pusterla *et al.* 2005; Dechant *et al.* 2006; Arnold *et al.* 2008; Conwell *et al.* 2010). In cases taken to surgery the source of haemorrhage was identified in 77.7% (Conwell *et al.* 2010).

Dechant *et al.* (2006) reported a survival rate of 44.7% at the time of discharge following medical management of HP however no specific treatment was significantly associated with survival. Supportive medical care may include i.v. fluid therapy, blood transfusions, nonsteroidal anti-inflammatories and antifibrinolytics (Dechant *et al.* 2006; Conwell *et al.* 2010; Mudge 2014; Gray *et al.* 2015).

Surgical removal of GCTs is the only solution available for treatment of the typical presentation of aggressive, stallion-like or nymphomania behaviour, infertility and anoestrous (McCue *et al.* 2006; Sherlock *et al.* 2016). Laparotomy (Crabtree 2011) and laparoscopy have both been reported for surgical removal of large, nonruptured GCTs (Rodgers *et al.* 2002; de Bont *et al.* 2010). Laparoscopic techniques are currently favoured as they are minimally invasive and allow improved visualisation of the ovarian pedicle for ligation (Hubert *et al.* 2006; Lloyd *et al.* 2007; de Bont *et al.* 2010), and standing laparoscopic ovariectomy is associated with a lower post-operative complication rate versus conventional surgery in mares (Lloyd *et al.* 2007; de Bont *et al.* 2010; Sherlock *et al.* 2016). Laparoscopic transection of the ovarian pedicle was performed in Case 2 24 h after abdominal drainage, however a significant volume of fluid was still present in the abdomen which impeded visualisation to a degree. Repeat abdominal ultrasound examination following drainage and ideally on the day of surgery could have been performed in Case 2 to gauge the remaining volume of HP. This would guide the surgeon as to whether laparoscopic visualisation of the ovarian pedicle was likely to be adequate or not. However, if the volume of HP is large or the case is unstable and therefore not a suitable candidate for standing sedation (as in Case 1), laparoscopy is very unlikely to be successful and should steer the surgical choice towards laparotomy.

There are many choices for surgical ligation of thickened ovarian pedicles including bipolar electrocautery with or without suturing, stapling of the mesovarium and the use of endoscopic clips (Doran *et al.* 1988; Lloyd *et al.* 2007; Smith and Mair 2008; de Bont *et al.* 2010; Kummer *et al.* 2010; Goodin *et al.* 2011). Whichever method is used, care must be taken that haemostasis is complete and secure.

Surgical removal of GCTs using a two-step procedure (laparoscopic transection of the ovarian pedicle followed by removal via ventral midline laparotomy) has been reported for removal of very large ovaries (up to 50 cm in size) (Vitte *et al.* 2014), and this was the surgical method performed in Case 2. Retrieval of very large tumours via a ventral midline laparotomy may be advantageous over making a very large flank laparotomy incision in a standing case and lessened post-operative complications (Vitte *et al.* 2014). However, there is the additional associated risk and cost of general anaesthesia to consider. Alternatively, aspiration of the fluid contents of the mass or further dissection of enlarged ovaries with a morcellator into smaller size pieces in a specimen retrieval bag via a flank incision has been reported (de Bont *et al.* 2010; Kummer *et al.* 2010), but we believe that this would not have been a realistic option in our two cases due to the size of the ovaries.

Prognosis

There is a good prognosis following standing laparoscopic removal of large, pathological ovaries and low complication rates are reported: 99–100% of cases are reported to return to their previous level of work and 85–93% of cases are

successfully bred within 30 months after surgery (Hubert *et al.* 2006; Lloyd *et al.* 2007; de Bont *et al.* 2010; Kummer *et al.* 2010; Röcken *et al.* 2011; Kelmer *et al.* 2013). Short-term survival rates for HP vary from 39 to 74% (Pusterla *et al.* 2005; Dechant *et al.* 2006; Conwell *et al.* 2010). Of the HP cases taken to surgery, a 42% survival rate has been reported (Conwell *et al.* 2010) and surgical treatment of GCTs associated with HP was curative in all cases (Alexander *et al.* 2004; Dechant *et al.* 2006).

Conclusion

In summary, GCTs should be considered as a differential diagnosis in mares presenting with HP. In some cases, the intra-abdominal haemorrhage is acute and severe and may necessitate emergency resuscitation of the case. Medical stabilisation of the case is initially recommended and surgical removal of the affected ovary is likely to result in a successful outcome.

Authors' declaration of interests

No conflicts of interest have been declared.

Ethical animal research

This is a retrospective case report and as such no research animals were involved and ethical review was not applicable. Both cases were client owned, informed owner consent was obtained for treatment and publication. Client confidentiality has been maintained.

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Authorship

F. Worsman assisted with management of Case 1 and wrote the majority of the manuscript. S. Barakzai managed Case 2, prepared Case 2 details for the manuscript and edited the manuscript. M. de Bont and L. Rubio-Martinez managed Case 1 and edited the manuscript. S. Turner assisted with management of Case 2. All authors approved the final manuscript.

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¹GE Medical Systems Limited, Chalfont St Giles, Buckinghamshire, UK.

²Boehringer Ingelheim Limited, Bracknell, Berkshire, UK.

³Animalcare, York, UK.

⁴Pfizer, Tadworth, Surrey, UK.

⁵Covidien Surgical Solutions, Dublin, Ireland.

⁶Fresenius Kabi, Runcorn, Cheshire, UK.

⁷Dechra, Northwich, Cheshire, UK.

⁸Intervet, Milton Keynes, Buckinghamshire, UK.

⁹Norbrook, Corby, Northamptonshire, UK.

¹⁰Hameln Pharmaceuticals, Gloucester, UK.

¹¹Sims Portex Ltd, Hythe, Kent, UK.

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